Pushing the Boundaries of High Sensitivity Epigenetic Applications

Discover the power of the Twist NGS Methylation Detection System, alongside the unique enzymatic conversion process from New England Biolabs®. Read this webinar breakdown, examine the benefits of the Twist pre-capture conversion probe design approach, and see how this workflow leads to increased confidence in methylated-base calls in clinically relevant sample types.

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Twist Bioscience originally aired the webinar highlighted in this article. View the original webinar at this link.

Introduction

Epigenetics, the study of changes in gene activity that do not involve DNA sequence modifications, has become an important area of research in recent years. Researchers have used the technology to effectively advance early cancer detection, improve our scientific understanding of cellular growth, and unravel several areas of neurobiology. Methylation is one key area of epigenetics that researchers have focused on extensively.

DNA methylation is an essential epigenetic modification involving the addition of a methyl (-CH_3) group onto the C5 position of a cytosine base. The resulting 5-methylcytosine affects the control of gene expression. Anomalies in this process, such as the hypo- or hypermethylation of cytosine-guanine (CpG) dinucleotides, can lead to genomic instabilities or issues with transcriptional silencing. These effects can result in various diseases – including cancer, diabetes, cardiovascular disease, and various inflammatory conditions. Importantly, these changes can happen in the first stages of cancer development, so accurately detecting them opens the doors for effective early cancer detection. Thus, scientists must access the right tools to effectively study a given sample's DNA methylation status and better understand genome-wide methylation patterns.

A new era in epigenetic research

Researchers typically use a methylation-sequencing (methyl-seq) method that treats DNA with bisulfite salts to convert unmethylated cytosine residues into uracil. These then get translated into thymine during amplification, meaning only methylated cytosines show up in sequencing data. By comparing the treated DNA against the untreated DNA, scientists can determine the methylation status of the sample in question.

However, bisulfite treatment is harsh, and DNA is degraded in the sample, leading to loss of coverage and missed methylated sites. Twist Bioscience has developed the Twist Methylation Detection System, which uses an alternative workflow to circumvent this issue and maximize the identification of methylated cytosines. The workflow employs A unique enzymatic DNA conversion process from New England Biolabs®, alongside Twist's Custom Methylation Panel design capabilities. In order to increase NGS efficiency in the workflow, target capture is used to isolate only potentially methylated regions of interest in a sample. Also, enzymatic conversion causes considerably less damage to the DNA than bisulfite conversion treatments.

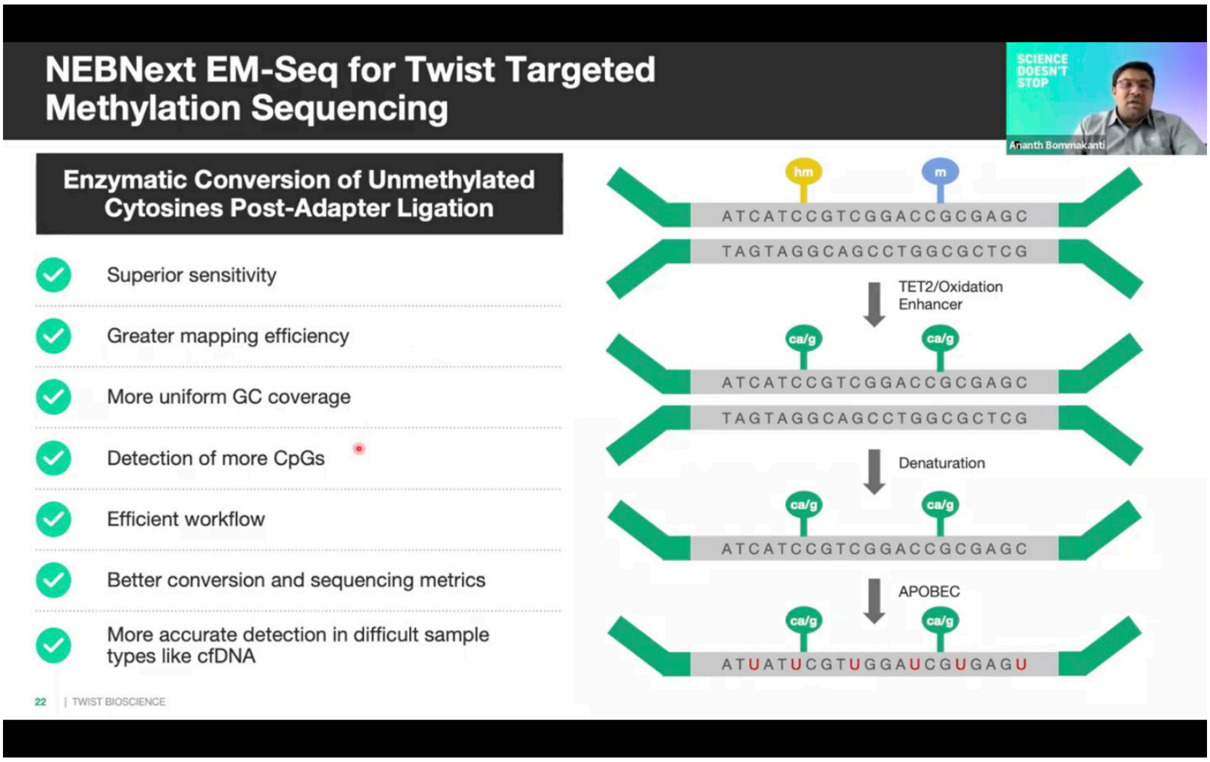
As methyl-seq is an essential tool for detecting various disorders like cancer, it must also be compatible with sample types that provide tiny amounts of input DNA like liquid biopsy. As the relevant DNA is present in minute concentrations in a patient's blood sample, it must be amplified for target capture to be sensitive enough. However, DNA amplification removes methyl groups from cytosines. Twist's scientists developed a compatible workflow that converts unmethylated cytosines before target capture to support lower amounts of input sample DNA.

Pre-capture conversion has one main challenge. A significant number of target regions are modified, and the sample has its complexity significantly decreased. The design of the target capture probe panel needs to handle this decreased complexity and avoid both off-target capture and target drop-out. Twist employs advanced AI-based design algorithms to address this challenge by designing custom probes that can take this pre-capture conversion into account and capture all the methylated and unmethylated parts of the target region effectively. The result is a probe panel with unmatched sensitivity that offers researchers increased confidence in methylated-base calls, even for low input volume samples like liquid biopsy.

Deep dive: How enzymatic conversion leads to better methyl-seq results

As discussed, the bisulfite chemical conversion process is harsh and can damage the DNA in a sample, which is especially problematic for low input volume sample types. Consequently, data quality is often severely impacted, and samples prepared in this way may require deeper sequencing efforts or not give satisfactory results.

Enzymatic conversion from New England Biolabs® utilizes two enzymes that, when used together, mimic bisulfite conversion with considerably less damage to the DNA. The process combines one enzyme that protects the hydroxymethylcytosines and methylcytosines, denaturing the sample, and another that converts all of the remaining unmodified cytosines to uracil. Just as with bisulfite conversion, the subsequent PCR amplification step will convert uracil into thymine, leaving methylated cytosines for detection during sequencing analyses.



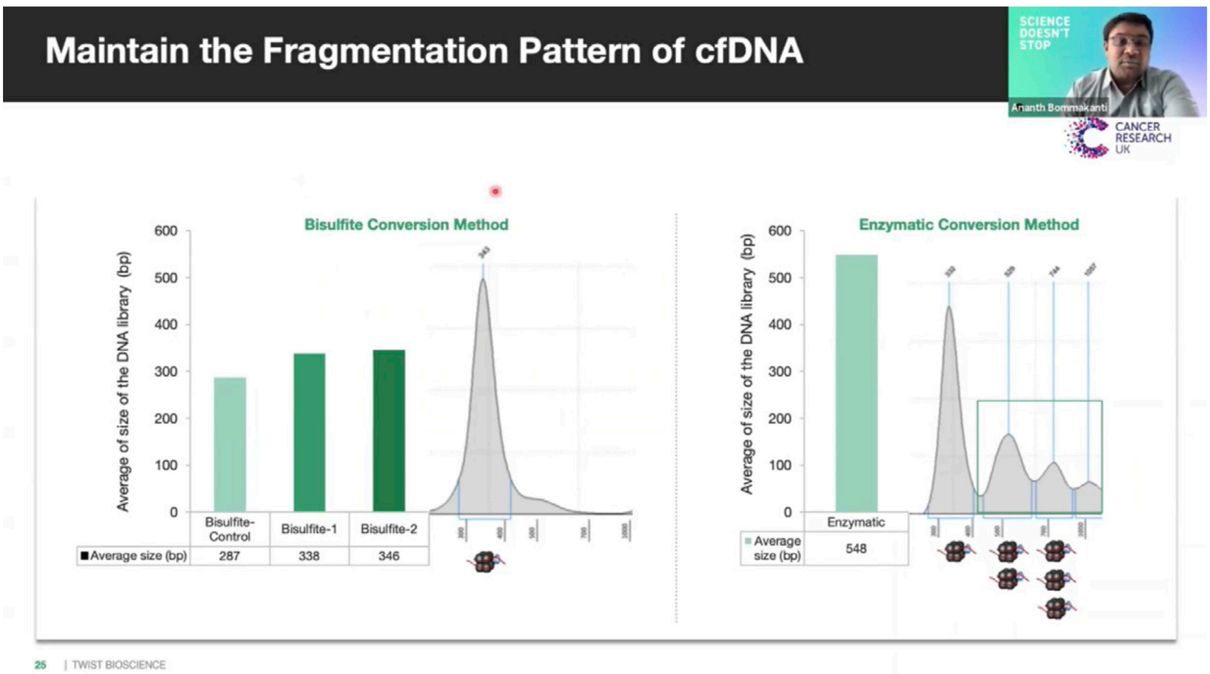
Also contained in Twist Bioscience's Methylation Detection kit are two control samples: an unmethylated lambda DNA sample and a CpG methylated DNA sample. These allow researchers to measure the conversion efficiencies of the system. Twist demonstrated that the enzymatic conversion method achieved a conversion efficiency of over 99.5% for both samples, higher than what researchers typically observe in bisulfite workflows. Such high efficiency is advantageous as it reduces the likelihood of false-positive results. The enzymatic conversion method can also detect converted individual cytosines with comparable performance to the bisulfite method and covers on average more than 15% more CpGs than bisulfite methods.

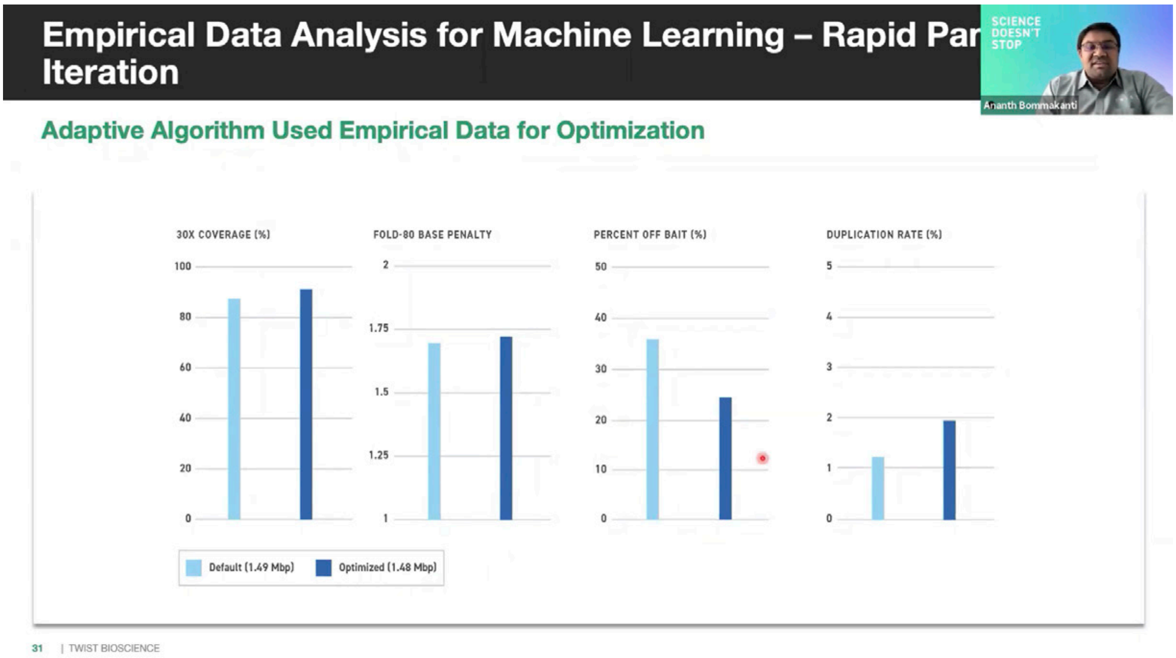
When studying circulating free DNA, there will be traces of high-molecular-weight fragments in the data resulting from the histone positioning in the DNA. These positioning markers are crucial in differentiating between cancerous and non-cancerous states in early cancer detection, so retaining and analyzing these high molecular weight fragments is essential. Bisulfite conversion obscures high molecular weight fragments. However, enzymatic conversion can maintain this fragmentation pattern, making this technique specifically advantageous to cancer epigenetics research.

Deep dive: How target enrichment probe design improves Methyl-seq

An enzymatic conversion performed before target capture ensures minimal sample loss, which is particularly advantageous for samples with a low input volume, and is especially important for successful early cancer detection. However, this method significantly decreases sample complexity due to the conversion of most of the cytosines. Hybridization capture faces two challenges during a pre-capture workflow: potential loss of regions that undergo significant change, and off-target binding of probes to low complexity regions.

Twist Bioscience has developed advanced probe design algorithms to circumvent these issues. Each custom methyl-seq panel will contain four probes per target region. These include probes that capture both the sense and antisense DNA strands, and both the methylated and unmethylated forms of the sequence. A machine learning algorithm analyses the combination of targets and fine-tunes this design process to minimize off-target effects across all genomic outcomes based on a level of stringency that the user sets. For example, the algorithm may optimize the target by removing target sequence regions with low complexity or extreme repetition. In exchange for around 0.01 Mpb in targets, the optimization dramatically





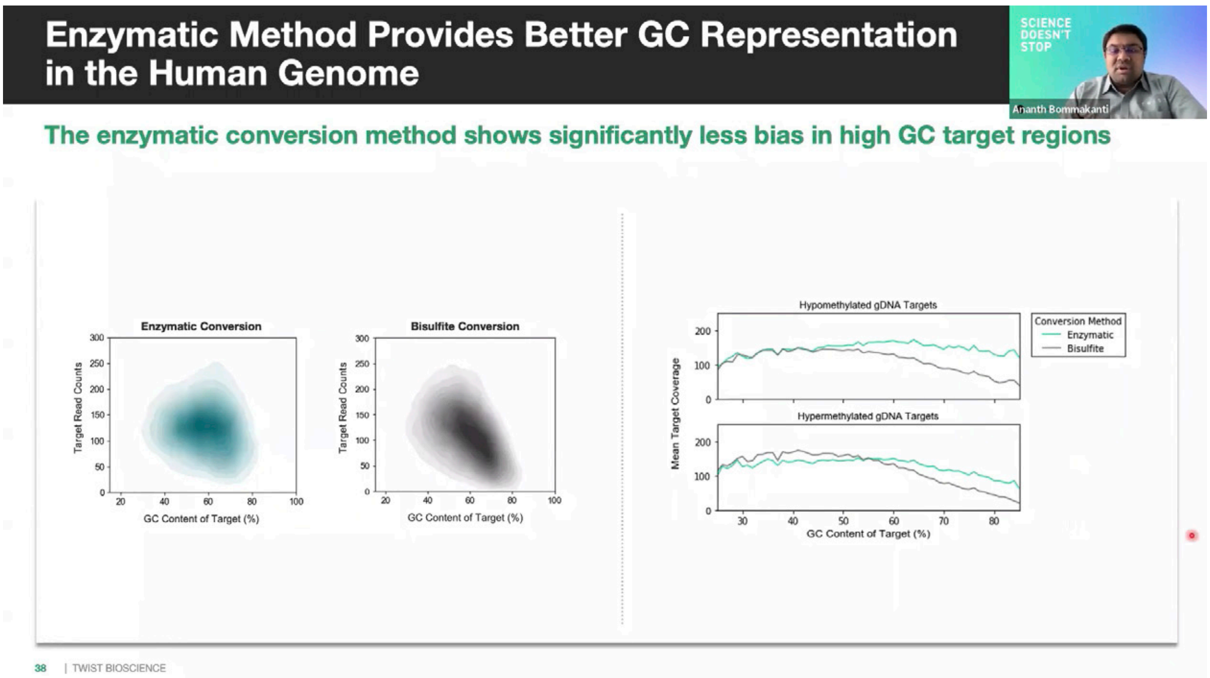
lowers the incidence of off-target effects and slightly improves the 30x coverage.

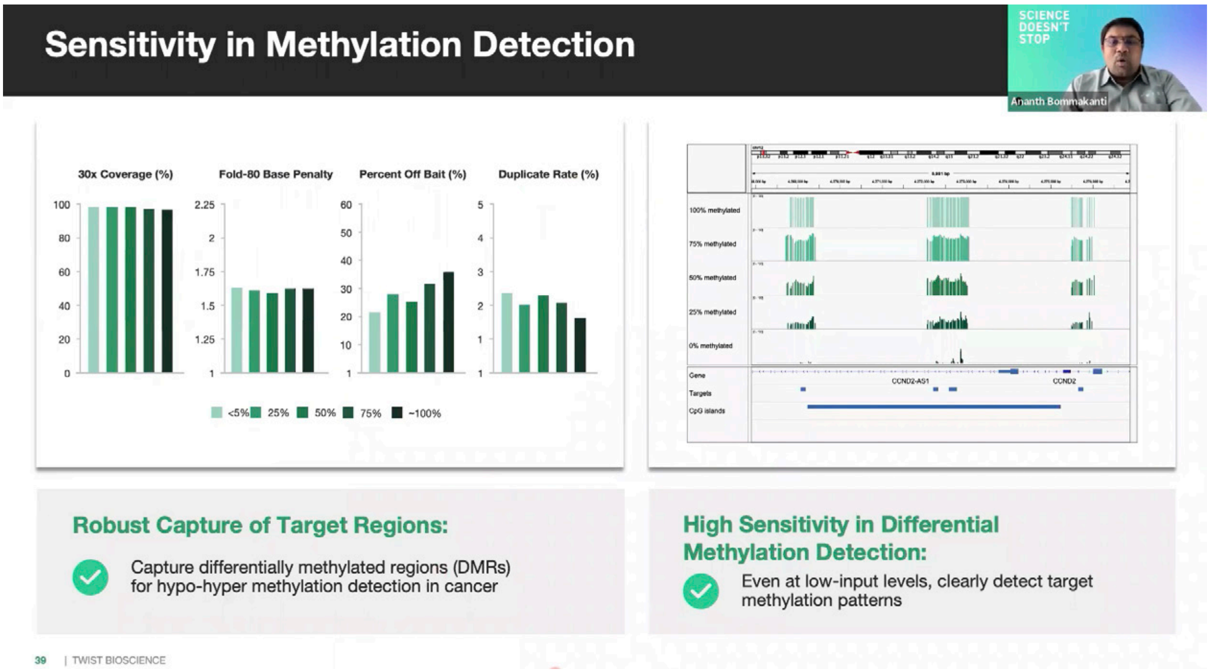
Twist also offers a unique and innovative methylation enhancer that can further improve the off-target ratio. This methyl-seq specific blocker is added during target capture to reduce the occurrence of off-target effects further without negatively affecting any key metric, including uniformity, 30x coverage, and duplication rate.

By optimizing the target capture process, the Twist Methylation Detection System can return better sequencing data and ultimately save on sequencing costs.

Superior performance in action

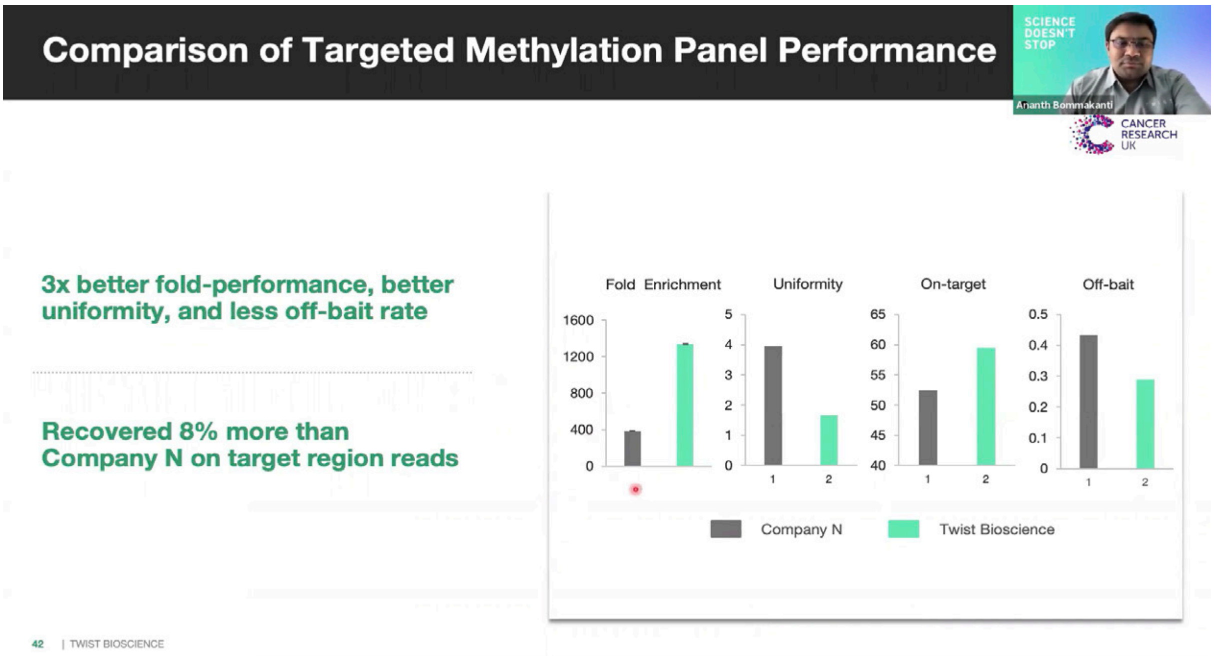
The Twist Next Generation Sequencing (NGS) Methylation Detection System offers superior

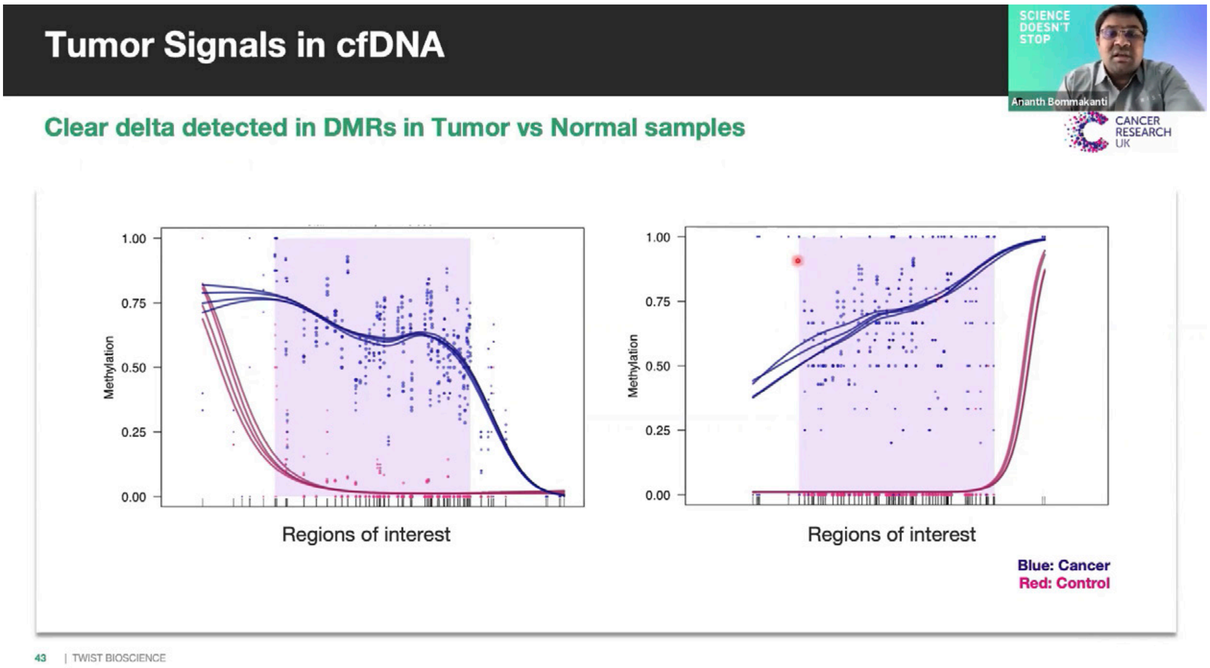




performance compared to other methods and other industry competitors. Due to probe design optimizations, the workflow offers reduced bias in high GC target regions compared to bisulfite conversion. Such hypermethylated regions are often a key focus in epigenetics, and so if the conversion method cannot capture these regions clearly without bias, there is a risk that data will be misinterpreted or lost.

The Twist NGS Methylation Detection System is also highly sensitive. Experimental data from cancer cell lines showed that the Twist system could capture differentially methylated regions (DMRs) with a high degree of success. Additionally, when testing the Twist system against multiple cell lines with differing degrees of methylation in a target genomic region, the system demonstrated robust differentiation between samples.





Twist scientists also tested an unoptimized, out-of-the-box Twist panel against an optimized panel from an anonymous market competitor in order to demonstrate the performance of the Twist Methylation Detection System. In these tests, the Twist system had a 3-fold increase in fold enrichment performance and a similarly significant advantage in the uniformity of reads. The Twist system's on-target rate is considerably higher while still reporting a reduction of between 15-20% in off-target effects. In addition, the Twist system recovered 8% more on target reads than the anonymous competitor system.

Finally, One of Twist's research partners sought to understand the performance of the Twist system when used to detect cancerous biomarkers in cfDNA. In order to be effective, the system must be able to demarcate between the methylation patterns in standard samples and tumor samples with high levels of sensitivity so that researchers can identify healthy, early cancer, and metastasized cancer states using patterns of methylation. When tested using control samples and tumor samples, the Twist system could differentiate the two samples according to methylation status in the cfDNA. All together, the Twist Methylation Detection workflow is a powerful tool for studying the links between methylation and early cancer detection.

Conclusion

The Twist NGS Methylation Detection System combines a unique enzymatic conversion process from New England Biolabs® and Twist Bioscience's Custom Methylation panels for a robust, end-to-end sample preparation and analysis solution for the efficient identification of methylated genomic DNA. The workflow results in significantly less damage to DNA than the current gold-standard chemical conversion process, leading to superior sensitivity, more uniform GC mapping coverage, and better conversion and sequencing metrics. Twist's Custom Methylation Panels are intelligently designed to drive high-performance enrichment. Through its efficient methylation detection, the Twist NGS Methylation Detection System pushes the boundaries of high sensitivity epigenetic applications.